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(54) Title: PROKARYOTIC EXPRESSION OF MHC PROTEINS

(57) Abstract

The present invention is directed to unglycosylated, prokaryotically-expressed MHC polypeptides, methods of producing these polypeptides, and complexes consisting essentially of an isolated MHC component and an antigenic peptide associated with the antigen binding site of the MHC component. These complexes are useful in treating deleterious immune responses, such as autoimmunity.

# PROKARYOTIC EXPRESSION OF MHC PROTEINS

The present application is a continuation-in-part of U.S.S.N. 08/143,575 filed October 25, 1993. The entire application is incorporated herein by reference.

## BACKGROUND OF THE INVENTION

The invention relates to methods of producing compositions for the modulation of T cell function in the treatment of for example, autoimmune diseases, allergic responses, transplant rejection, and other immunological disorders. In particular, it concerns production of major histocompatibility complex (MHC) class I and class II proteins in prokaryotes that have been transformed with nucleotide sequences that code for the proteins. The MHC proteins are useful for making complexes that target T cells. The complexes comprise the MHC proteins and peptides representing fragments of antigens associated with the particular diseases. These complexes can be further conjugated to radioisotopes or other labels for diagnostic purposes, or to toxins or other substances which render the complexes therapeutically useful.

A number of pathological responses involving unwanted T cell activation are known. For instance, a number of allergic diseases have been associated with particular MHC alleles or are suspected of having an autoimmune component.

Other deleterious T cell-mediated responses include the destruction of foreign cells that are purposely introduced into the body as grafts or transplants from allogeneic hosts. This process, known as "allograft rejection," involves the interaction of host T cells with foreign MHC molecules. Quite often, a broad range of MHC alleles are involved in the response of the host to an allograft.

Autoimmune disease is a particularly important class of deleterious immune response. In autoimmune diseases, self-tolerance is lost and the immune system attacks "self" tissue as if it were a foreign target. More than 30 autoimmune diseases are presently known; these include many which have received much public attention, including myasthenia gravis (MG) and multiple sclerosis (MS).

The involvement of the MHC Class II proteins in autoimmune disease has been shown in animal models. Administration of antibodies to either MHC Class II proteins themselves or antibodies to agents that induce expression of the MHC Class II

The present invention includes a method of producing an MHC polypeptide comprising the steps of (a) growing in a culture prokaryotic cells containing an expression vector comprising a nucleotide sequence encoding the MHC polypeptide under such conditions that the polypeptide is expressed; and (b) extracting and isolating the MHC polypeptide. The method provides for the expression of two MHC polypeptides in a single prokaryotic cell, wherein the polypeptides form a heterodimer. The compositions produced by the method are also the subject of the invention.

The invention further provides for a prokaryotic expression vector comprising a nucleotide sequence coding for an MHC polypeptide operably linked to a prokaryotic promoter sequence. A signal sequence operably liked to the MHC polypeptide sequence is optionally included in the vector. The nucleotide sequence for the MHC polypeptide can encode a truncated MHC polypeptide, or an MHC polypeptide lacking a transmembrane domain, in addition to full-length MHC polypeptides and other constructs which are derived from the full-length MHC polypeptide. The vector can be used to transform a prokaryotic cell such as *E. coli*.

The invention additionally provides for a substantially pure MHC-peptide complex consisting essentially of an antigenic peptide and an isolated recombinant MHC component having altered glycosylation and an antigen binding site, wherein the antigenic peptide is associated with the antigen binding site. The peptide is typically between about 8 and about 30 amino acids, but can be shorter or longer. The peptide can be noncovalently associated with the antigen binding site. The present invention includes peptides which are autoantigenic and thereby associated with an autoimmune disease. An epitope on the peptide can be recognized, for example, by an autoreactive T cell associated with multiple sclerosis, rheumatoid arthritis, or myasthenia gravis. Suitable peptide include those comprising residues 138-167 of human AChR $\alpha$ subunit, residues 84-102 of human MBP, and residues 148-162 of human MBP.

Also provided are pharmaceutical compositions comprising a pharmaceutically acceptable carrier and the recombinant MHC-peptide complex. An example of the pharmaceutical composition includes a composition in which the MHC-peptide complex is embedded in a liposome.

Definitions

A "nucleotide sequence encoding an MHC polypeptide" is a subsequence or full length polynucleotide sequence which, when present in a cell, expresses an MHC

*Sci. (U.S.A.)* 85: 2444 (1988), and by computerized implementations of these algorithms. Typically, the program providing the highest percentage identity is used.

"Percentage of sequence identity" is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has at least 70% sequence identity, preferably at least 80%, more preferably at least 90% and most preferably at least 95%, compared to a reference sequence using the programs described above using standard parameters. One of skill will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of at least 70%, preferably at least 80%, more preferably at least 90%, and most preferably at least 95%.

Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other under stringent conditions. Stringent conditions are sequence dependent and will be different in different circumstances. Generally, stringent conditions are selected to be about 5° C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is at least about 0.02 molar at pH 7 and the temperature is at least about 60°C.

Another indication that protein sequences are substantially identical is if one protein is immunologically reactive with antibodies raised against the other protein.

The unglycosylated recombinant MHC polypeptides of the invention are typically produced by a prokaryotic host cell that has been transformed with a nucleotide sequence that encodes the MHC polypeptide. Recombinant DNA techniques are generally used to link the MHC-encoding nucleotide sequence to signals that control gene expression. As a consequence of being produced in prokaryotic host cells, the MHC polypeptides lack the carbohydrate moieties that are normally found on MHC polypeptides from eukaryotic cells.

## BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows kinetics of peptide binding to recombinant DR2 chains expressed in *E. coli*.

Figure 2 shows the stability of recombinant DR2-peptide complexes.

Figure 3 shows optimum pH for maximum binding of MBP peptids to purified recombinant DR2 polypeptide chains.

Figure 4 shows γIFN production in T cells contacted with complexes of the invention.

Figure 5 shows γIFN production in T cells contacted with complexes of the invention.

Figure 6 shows efficacy of complexes of the invention in an animal model for multiple sclerosis.

## DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention provides recombinant MHC polypeptides that can be used to form complexes useful for modulating T cell function, and methods for producing the MHC polypeptides. The complexes, which consist of the MHC polypeptides complexed with antigenic peptides, can be used to inhibit a deleterious T cell-mediated immune response, such as allergic responses, allograft rejection, and autoimmune diseases. In addition, the complexes can be used to promote immune responses and can be used as vaccines.

The present invention also provides methods for producing MHC proteins on a commercially viable scale. A further advantage provided by the present invention is that it provides a readily adaptable means of producing modified MHC polypeptides that are useful for a variety of desired uses. For example, when using the complexes for

MHC class II chains to bind and present peptide. The alpha and beta chains of seven allelic variants of the I-A region have been cloned and sequenced (Estees *et al.*, "T cell Clones" in *Regulation of Immune Gene Expression*, Feldman *et al.*, eds. (Humana Press 1985), pp. 3-19. Methods for purifying the murine I-A (Class II) histocompatibility proteins have been disclosed by Turkewitz, A.P., *et al.*, *Molecular Immunology* (1983) 20: 1139-1147. These methods, which are also suitable for Class I molecules, involve preparation of a soluble membrane extract from cells containing the desired MHC molecule using nonionic detergents, such as NP-40, Tween 80 and the like. The MHC molecules are then purified by affinity chromatography, using a column containing antibodies raised against the desired MHC molecule. Use of 0.02% Tween-80 in the elution buffer is helpful to eliminate aggregation of the purified molecules.

The human Class I proteins have also been studied. The MHC of humans (HLA) on chromosome 6 has three loci, HLA-A, HLA-B, and HLA-C, the first two of which have a large number of alleles encoding alloantigens. These are found to consist of a 44 kd subunit and a 12 kd $beta_2$-microglobulin subunit which is common to all antigenic specificities. Isolation of these detergent-soluble HLA antigens was described by Springer, T.A., *et al.*, *Proc. Natl. Acad. Sci. USA* (1976) 73: 2481-2485; Clementson, K.J., *et al.*, in "Membrane Proteins" Azzi, A., ed; Bjorkman, P., Ph.D. Thesis Harvard (1984).

Further work has resulted in a detailed picture of the 3-D structure of HLA-A2, a Class I human antigen. (Bjorkman, P.J., *et al.*, *Nature* (1987) 329: 506-512, 512-518. In this picture, the $\beta_2$-microglobulin protein and $alpha_3$ segment of the heavy chain are associated; the $alpha_1$ and $alpha_2$ regions of the heavy chain appear to form antigen-binding sites to which the peptide is bound (*Science* (1987) 238:613-614, Bjorkman, P.J. *et al. Nature* (*supra*). Soluble HLA-A2 can be purified after papain digestion of plasma membranes from the homozygous human lymphoblastoid cell line J-Y as described by Turner, M.J. *et al.*, *J. Biol. Chem.* (1977) 252: 7555-7567. Papain cleaves the 44 kd chain close to the transmembrane region yielding a molecule comprised of $alpha_1$, $alpha_2$, $alpha_3$, and $\beta_2$ microglobulin.

The three dimensional structure of human Class II MHC antigens has also been determined and is similar to that of of Class I molecules. Antigenic peptides are bound in an open ended antigen binding groove. The binding groove is formed from the N-terminal domain portions of two class II chains which extend from the membrane

large segments of genomic DNA are generated by random fragmentation and are ligated with vector DNA to form concatemers that can be packaged into the appropriate vector. Two kinds of vectors are commonly used for this purpose, bacteriophage lambda vectors and cosmids.

To prepare cDNA, mRNA from the organism of interest is first isolated. Eukaryotic mRNA has at its 3' end a string of adenine nucleotide residues known as the poly-A tail. Short chains of oligo d-T nucleotides are then hybridized with the poly-A tails serving as a primer for the enzyme reverse transcriptase. This enzyme uses RNA as a template to synthesize a complementary DNA (cDNA) strand. A second DNA strand is then synthesized using the first cDNA strand as a template. Linkers are added to the double-stranded cDNA for insertion into a plasmid or λ phage vector for propagation in *E. coli*.

Identification of clones in either genomic or cDNA libraries harboring the desired nucleic acid segments is performed by either nucleic acid hybridization, or immunological detection of the encoded protein if an expression vector is used. The bacterial colonies are then replica plated on solid support, such as nitrocellulose filters. The cells are lysed and probed with either oligonucleotide probes described above or with antibodies to the desired protein.

Other methods well known to those skilled in the art can also be used to identify desired genes. For example, amplification techniques, such as the polymerase chain reaction (PCR) can be used to amplify the desired nucleotide sequence. U.S. Patents Nos. 4,683,195 and 4,683,202 describe this method. Sequences amplified by PCR can be purified from agarose gels and cloned into an appropriate vector according to standard techniques.

Prokaryotic Expression of MHC Polypeptides

Prokaryotes that are useful as host cells, according to the present invention, most frequently are represented by various strains of *E. coli*. However, other microbial strains can also be used, such as bacilli, for example *Bacillus subtilis*, various species of *Pseudomonas*, or other bacterial strains.

According to the invention, the MHC polypeptides are expressed from cloned nucleotide sequences that encode the MHC polypeptides by operably linking the truncated or full-length nucleic acids to signals that direct gene expression in prokaryotes. A nucleic acid is "operably linked" when it is placed into a functional

lactamase (penicillinase) and lactose (*lac*) promoter systems (Change *et al.*, *Nature* (1977) 198: 1056) and the tryptophan (*trp*) promoter system (Goeddel *et al.*, *Nucleic Acids Res.* (1980) 8: 4057) and the lambda-derived $P_L$ promoter and N-gene ribosome binding site (Shimatake *et al.*, *Nature* (1981) 292: 128). Any available promoter system that functions in prokaryotes can be used.

Either constitutive or regulated promoters can be used in the present invention. Regulated promoters can be advantageous because the host cells can be grown to high densities before expression of the MHC polypeptides is induced. High level expression of heterologous proteins slows cell growth in some situations. Regulated promoters especially suitable for use in *E. coli* include the bacteriophage lambda $P_L$ promoter, the hybrid *trp-lac* promoter (Amann *et al.*, Gene (1983) 25: 167; de Boer *et al.*, *Proc. Natl. Acad. Sci. USA* (1983) 80: 21, and the bacteriophage T7 promoter (Studier *et al.*, *J. Mol. Biol.* (1986); Tabor *et al.*, (1985). These promoters and their use are discussed in Sambrook *et al.*, *supra.*

For expression of MHC polypeptides in prokaryotic cells other than *E. coli*, a promoter that functions in the particular prokaryotic species is required. Such promoters can be obtained from genes that have been cloned from the species, or heterologous promoters can be used. For example, the hybrid *trp-lac* promoter functions in *Bacillus* in addition to *E. coli.*

A ribosome binding site (RBS) is also necessary for expression of MHC polypeptides in prokaryotes. An RBS in *E. coli*, for example, consists of a nucleotide sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon (Shine and Dalgarno, *Nature* (1975) 254: 34; Steitz, *In Biological regulation and development: Gene expression* (ed. R.F. Goldberger), vol. 1, p. 349, 1979, Plenum Publishing, NY).

Translational coupling may be used to enhance expression. The strategy uses a short upstream open reading frame derived from a highly expressed gene native to the translational system, which is placed downstream of the promoter, and a ribosome binding site followed after a few amino acid codons by a termination codon. Just prior to the termination codon is a second ribosome binding site, and following the termination codon is a start codon for the initiation of translation. The system dissolves secondary structure in the RNA, allowing for the efficient initiation of translation. See Squires, et. al. (1988), *J. Biol. Chem.* 263: 16297-16302.

A preferred system for obtaining recombinant proteins from *E. coli* which maintains the integrity of their N-termini has been described by Miller *et al.* *Biotechnology* 7:698-704 (1989). In this system, the gene of interest is produced as a C-terminal fusion to the first 76 residues of the yeast ubiquitin gene contianing a peptidase cleavage site. Cleavage at the junction of the two moieties results in production of a protein having an intact authentic N-terminal reside.

The vectors containing the nucleic acids that code for the MHC polypeptide are transformed into prokaryotic host cells for expression. "Transformation" refers to the introduction of vectors containing the nucleic acids of interest directly into host cells by well known methods. The particular procedure used to introduce the genetic material into the host cell for expression of the MHC polypeptide is not particularly critical. Any of the well known procedures for introducing foreign nucleotide sequences into host cells may be used. It is only necessary that the particular procedure utilized be capable of successfully introducing at least one gene into the host cell which is capable of expressing the gene.

Transformation methods, which vary depending on the type of host cell, include electroporation; transfection employing calcium chloride, rubidium chloride calcium phosphate, or other substances; microprojectile bombardment; infection (where the vector is an infectious agent); and other methods. *See, generally*, Sambrook *et al.*, (1989) *supra*, and *Current Protocols in Molecular Biology*, *supra*. Reference to cells into which the nucleic acids described above have been introduced is meant to also include the progeny of such cells. Transformed prokaryotic cells that contain expression vectors for expressing MHC polypeptides are also included in the invention.

After standard transfection or transformation methods are used to produce prokaryotic cell lines that express large quantities of the MHC polypeptide, the polypeptide is then purified using standard techniques. *See, e.g.*, Colley *et al.* (1989) *J. Biol. Chem.* 64: 17619-17622; and *Methods in Enzymology*, "Guide to Protein Purification", M. Deutscher, ed. Vol. 182 (1990). The recombinant cells are grown and the MHC polypeptide is expressed. The purification protocol will depend upon whether the MHC polypeptide is expressed intracellularly, into the periplasm, or secreted from the cell. For intracellular expression, the cells are harvested, lysed, and the MHC polypeptide is recovered from the cell lysate (Sambrook *et al.*, *supra.*). Periplasmic MHC polypeptide is released from the periplasm by standard techniques (Sambrook *et*

polypeptides consist essentially of either the $\alpha_1$ or $\beta_1$ domain from the full-length polypeptide. Such fragments typically comprise between about 50 and about 100 amino acids, preferably between about 60 and about 90, more preferably between about 70 and about 80. Alternatively, synthetic methods may be used to prepare polypeptides. *See, e.g.*, Merrifield (1986) *Science* 232: 341-347; Atherton *et al.*, *Solid Phase Peptide Synthesis: A Practical Approach*, IRL Press, Oxford).

In general, modifications of the sequences encoding the MHC polypeptides is readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis (*see*, Gillman and Smith (1979) *Gene* 8: 81-97, and Roberts, S. *et al.* (1987) *Nature* 328: 731-734). Most modifications are evaluated by routine screening in a suitable assay for the desired characteristic. For instance, the effect of various modifications on the ability of the polypeptide to bind peptide or affect T-cell proliferation can be easily determined using the assays described below. Modifications of other properties such as redox or thermal stability, hydrophobicity, susceptibility to proteolysis, or the tendency to aggregate are all assayed according to standard techniques.

For certain applications, the MHC cDNA coding sequences are modified to delete the transmembrane domain and express the resulting soluble MHC polypeptides. Truncation of the MHC cDNA may be performed, for example, by oligonucleotide-directed deletion mutagenesis or polymerase chain reaction. Oligonucleotide-directed *in vitro* mutagenesis is described, for example, by Kunkel *et al.* (1987) *Meth. Enzymol.* 154: 367-382. *See also*, *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., Greene Publishing and Wiley-Interscience, New York (1987 and periodic supplements).

Pharmaceutical Use of MHC Polypeptides

The unglycosylated, prokaryotically-expressed MHC polypeptides of the invention can be used to form complexes with a peptide that represents an antigen associated with, for example, autoimmunity, allograft rejection or allergic responses. The components of the complex are chosen to have a desired effect on the immune system. An effective portion of an MHC polypeptide is one that comprises the antigen binding sites and sequences necessary for recognition of the MHC-peptide complex by the appropriate T cell receptor. The MHC component can be either a Class I or a Class

It is believed that the presentation of antigen by the MHC glycoprotein on the surface of antigen-presenting cells (APCs) occurs subsequent to the hydrolysis of antigenic proteins into smaller peptide units. The location of these smaller segments within the antigenic protein can be determined empirically. These segments are thought to be 8-18 residues in length, and contain both the agretope (recognized by the MHC molecule) and the epitope (recognized by T cell receptor on the T-helper cell). The epitope itself is a contiguous or non-contiguous sequence of 5-6 amino acids which recognizes the antigen-specific receptor of T-helper cells. The agretope is a continuous or non-contiguous sequence which is responsible for the association of the peptide with the MHC proteins.

The empirical process of determining the relevant 8-18 amino acid subunits is illustrated using the alpha subunit of the acetylcholine receptor of skeletal muscle. In myasthenia gravis (MG) an autoimmune response is directed to a region of this subunit. A loss of the acetyl choline receptors on the postsynaptic membrane of the neuromuscular junction causes the MG symptoms.

In MG, autoantibodies against the alpha subunit of the acetylcholine receptor (AChR) are associated with the autoimmune response directed at the AChR. Eighty five percent of MG patients have autoantibodies reactive with the alpha subunit. Of these, 60% have antibodies that bind to a peptide segment of the alpha subunit called the main immunogenic region (MIR) which is located between residues 60 and 80 (Tzartos and Lindstrom, *Proc. Natl. Acad. Sci. USA* (1980) 77: 755). The peptide segments recognized by autoreactive human T cells also are located on the alpha subunit (Hohlfeld *et al.*, *Proc. Natl. Acad. Sci. USA* (1987) 84: 5379-5383. The epitopes recognized by these T cells lie between residues 1-30, 125-147, 169-181, 257-271 and 351-368. In addition, in humans the AChR peptides 195-212 and 257-269 have been partially characterized as epitopes in myasthenia gravis patients of the HLA-DR5 and HLA-DR3, DQw2 MHC haplotypes, respectively (*see* Acha-Orbea (1989) *supra*).

The peptides carrying agretopes permitting presentation of the epitopes associated with alpha subunit of this receptor are readily determined. For example, determination of the appropriate peptides in a mouse model is carried out as follows.

Strains of mice which, when immunized with *Torpedo californicus* AChR develop a disease with many of the features of human myasthenia gravis, are used as model. MHC Class II glycoproteins are isolated from spleen cells of mice of this strain

peptides, applying standard stepwise solid phase peptide synthetic methods, followed by standard side chain deprotection and simultaneous release of the peptide amide from the solid support.

Alternatively the overlapping sequences which include the putative segments of 8-18 amino acids of the antigenic protein, such as acetylcholine receptor protein, can be synthesized on the method of Geysen, H.M., *et al. J. Immun. Meth.* (1987) 102: 274. The synthesized radiolabeled peptides are tested by incubating them individually (on the plates) with purified MHC proteins that have been formulated into lipid membrane bilayers as above.

In multiple sclerosis (MS), which results in the destruction of the myelin sheath in the central nervous system, myelin basic protein (MBP), the major protein component of myelin is the principal autoantigen. Pertinent segments of the MBP protein are also determined empirically, using a strain of mice which develops experimental allergic encephalitis (EAG) when immunized with bovine myelin basic protein.

Systemic lupus erythematosus (SLE) has a complex symptomology, but results from an autoimmune response to red blood cells. Peptides which are the antigenic effectors of this disease are found in the proteins on the surface of red blood cells.

Rheumatoid arthritis (RA) is a chronic inflammatory disease resulting from an immune response to proteins found in the synovial fluid.

Insulin-dependent diabetes mellitus (IDDM) results from autoimmune attack on the beta cells within the Islets of Langerhans which are responsible for secretion of insulin. Circulating antibodies to Islets cells surface antigens and to insulin are known to precede IDDM. Critical peptides in eliciting the immune response in IDDM are believed to be portions of the insulin sequence and the beta cell membrane surface proteins.

The relevant antigenic peptide subunits, as they are relatively short, can readily be synthesized using standard automated methods for peptide synthesis. In the alternative, they can be made recombinantly using isolated or synthetic DNA sequences, although this is not the most efficient approach for peptides of this length.

the autoimmune disease. The relevant complex should ameliorate or eliminate the symptoms of the disease.

Either of the types of complexes, *i.e.*, with or without the effector component, may be used. In one mode the treatment is two-fold. The individual is treated with the complex of prokaryotically-expressed, MHC-encoded antigen-presenting protein containing an effective portion of the antigenic peptide to down-regulate the immune system. Further down-regulation is achieved by treatment with the three component complex with includes the prokaryotically-expressed, MHC-encoded antigen-presenting protein, an effective portion of antigenic peptide which is specific for the autoimmune disease being treated, and an effector component. In addition, panels of complexes may be used for treatment. For example, if it is suspected that more than one peptide of an antigen is involved in the autoimmune response, and/or if it is suspected that more than one antigen is involved, the individual may be treated with several complexes selected from a panel containing the effective portion of the appropriate prokaryotically-expressed, MHC-encoded antigen-presenting polypeptides, and effective portions of antigenic peptides; these may be with or without effector components.

Administration of a labeled complex permits identification of those portions of the immune system involved in the disease, in diagnostic applications.

Selection of the MHC Complexes for Therapy and/or Diagnosis

In order to select the MHC complexes that are to be used in the diagnosis or treatment of an individual for a particular disease, the type of MHC antigens that are involved in the presentation of the antigen are identified. The following discussion describes the identification of antigen associated with autoimmune disease, but one of skill will recognize that the same general approach can be used for other diseases, such as allergies.

Specific autoimmune dysfunctions are correlated with specific MHC types. Methods for identifying which alleles, and subsequently which MHC encoded polypeptides, are associated with an autoimmune disease are known in the art. A method described in EP 286447 is suitable. In this method several steps are followed. First, the association between an MHC antigen and the autoimmune disease is determined based upon genetic studies. The methods for carrying out these studies are known to those skilled in the art, and information on all known HLA disease associations in humans is maintained in the HLA and Disease Registry in Copenhagen. The locus encoding the

treatment or diagnosis of an individual with rheumatoid arthritis would include those containing a polypeptide derived from the DR4(Dw4), DR1 and/or DR4(Dw14) which is capable of antigen presentation for disease induction, or incapable of antigen presentation for disease suppression, complexed with an effective portion of type-II collagen.

As used herein, the term "individual" encompasses all mammals and all vertebrates which possess basically equivalent MHC systems.

Formulation and Administration

If the transmembrane region of the MHC subunit is included, the complexes formed using prokaryotically-expressed MHC polypeptides of the invention are conveniently administered after being incorporated into lipid monolayers or bilayers. Typically liposomes are used for this purpose but any form of lipid membrane, such as planar lipid membranes or the cell membrane of a cell (*e.g.*, a red blood cell) may be used. The complexes are also conveniently incorporated into micelles. The data presented in Example 2, below, shows that MHC-peptide complexes comprising dimeric MHC molecules exist primarily as aggregates.

Liposomes can be prepared according to standard methods, as described below. However, if the transmembrane region is deleted, the complex can be administered in a manner conventionally used for peptide-containing pharmaceuticals.

Administration is systemic and is effected by injection, preferably intravenous, thus formulations compatible with the injection route of administration may be used. Suitable formulations are found in *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985). A variety of pharmaceutical compositions comprising complexes of the present invention and pharmaceutically effective carriers can be prepared. The pharmaceutical compositions are suitable in a variety of drug delivery systems. For a brief review of present methods of drug delivery, *see*, Langer, *Science* 249: 1527-1533 (1990).

In preparing pharmaceutical compositions using the prokaryotically-expressed, unglycosylated MHC polypeptides of the present invention, it is frequently desirable to modify the complexes of the present invention to alter their pharmacokinetics and biodistribution. For a general discussion of pharmacokinetics, *see*, *Remington's Pharmaceutical Sciences*, *supra*, Chapters 37-39. A number of methods for altering pharmacokinetics and biodistribution are known to one of ordinary skill in the art (*see*, *e.g.*, Langer, *supra*). For example, conjugation to soluble macromolecules, such as

subunits, phospholipids and/or surfactants. Thus, the micelles may comprise subunits and detergent, subunits in combination with both phospholipids and detergent, or subunits and phospholipid.

For pharmaceutical compositions which comprise the complexes of the present invention, the dose will vary according to, *e.g.*, the particular complex, the manner of administration, the particular disease being treated and its severity, the overall health and condition of the patient, and the judgment of the prescribing physician. Dosage levels for murine subjects are generally between about 10 μg and about 500 μg. A total dose of between about 50 μg and about 300 μg, is preferred. For instance, in treatments provided over the course of a disease, three 25 μg or 100 μg doses are effective. Total dosages range between about 0.015 and about 15 μg/kg, preferably about 0.15 to about 10 μg/kg.

The pharmaceutical compositions are intended for parenteral, topical, oral or local administration, such as by aerosol or transdermally, for prophylactic and/or therapeutic treatment. The pharmaceutical compositions can be administered in a variety f unit dosage forms depending upon the method of administration. For example, unit dosage forms suitable for oral administration include powder, tablets, pills, and capsules.

Preferably, the pharmaceutical compositions are administered intravenously. Thus, this invention provides compositions for intravenous administration which comprise a solution of the complex dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, *e.g.*, water, buffered water, 0.4% saline, and the like. For instance, phosphate buffered saline (PBS) is particularly suitable for administration of soluble complexes of the present invention. A preferred formulation is PBS containing 0.02% TWEEN-80. These compositions may be sterilized by conventional, well-known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on the severity of the disease and the weight and general state of the patient. As discussed above, this will typically be between about 0.5 mg/kg and about 25 mg/kg, preferably about 3 to about 15 mg/kg.

In prophylactic applications, the complexes of the invention are administered to a patient susceptible to or otherwise at risk of a particular disease. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts again depend on the patient's state of health and weight. The doses will generally be in the ranges set forth above.

In diagnostic applications, compositions containing the appropriate complexes or a cocktail thereof are administered to a patient suspected of having an autoimmune disease state to determine the presence of autoreactive T cells associated with the disease. Alternatively, the efficacy of a particular treatment can be monitored. An amount sufficient to accomplish this is defined to be a "diagnostically effective dose." In this use, the precise amounts will depend upon the patient's state of health and the like, but generally range from 0.01 to 1000 mg per dose, especially about 10 to about 100 mg per patient.

Kits can also be supplied for therapeutic or diagnostic uses. Thus, the complexes of the present invention may be provided, usually in a lyophilized form in a container. The complexes, which may be conjugated to a label or toxin, or unconjugated, are included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, *e.g.*, serum albumin, or the like, and a set of instructions for use. Generally, these materials will be present in less than about 5% wt. based on the amount of complex and usually present in total amount of at least about 0.001% wt. based again on the protein concentration. Frequently, it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1 to 99% wt. of the total composition. Where an antibody capable of binding to the complex is employed in an assay, this will usually be present in a separate vial. The antibody is typically conjugated to a label and formulated according to techniques well known in the art.

Unless defined otherwise, all technical and scientific terms used herein

pET3a was digested with *Eco*RI, blunt-ended with DNA Polymerase I (Klenow fragment) and digested with *Eco*RV. The vector was recircularized, destroying both restriction sites, to generate plasmid p26404.

Plasmid p26405 was derived from p26404 as follows. First, p26404 was digested with *Bam*HI and the ends were filled-in with DNA polymerase I (Klenow) to generate blunt ends. A synthetic linker of sequence: 5'...CGGAATTCCG...3' (SEQ. ID. No. 15) was introduced into the destroyed *Bam*HI site, thus replacing it with a new *Eco*RI site.

Plasmid p26411 was generated by digestion of p26405 with *Nde*I and *Eco*RI and insertion of a synthetic linker sequence:

5'...TATG GCT AGC ATG ACT GGT GGA CAG CAA ATG GGT-
3 '...AC CGA TCG TAC TGA CCA CCT GTC GTT TAC CCA-

-CGG GAT CCG AAGCTT AG...3' (SEQ. ID. No. 1)
-GCC CTA GGC TTCGAA TCTAA...5' (SEQ. ID. No.2)

This linker provides the first 14 codons of the phi-10 open reading frame (coupler), and has a *Bam*HI site located in the proper reading frame for subsequent expression of an inserted MHC gene. Downstream of the *Bam*HI site is a *Hin*dIII cloning site that together with the *Eco*RI site provides two downstream sites for directional cloning of inserts.

To facilitate cloning, one of the *Bam*HI sites in p26411 was destroyed to generate plasmid p27305. p26411 was digested with *Eco*RI + *Pst*I and the 3387bp fragment recovered. p26411 was also digested with *Bam*HI and *Pst*I and the 891bp fragment was recovered. A synthetic linker of sequence:

5'...AATTCCTACGTA...3'(SEQ. ID. No. 3)
3'...GGATGCATCTAG...5'(SEQ. ID. No. 4)

was made and ligated with the two fragments of p26411 to generate p27305. The linker has both *Eco*RI and *Bam*HI cohesive ends, but will regenerate only the *Eco*RI site upon ligation. Additionally, it carries a *Sna*BI (blunt) cloning site for future downstream manipulations.

Plasmid p27313 incorporates a copy of the *lacI*$^q$ repressor protein onto p27305 to control unwanted transcription of the target gene prior to induction. p27305 was digested with *Bam*HI and *Pst*I and a 917 bp fragment recovered. Plasmid pET11b

5'...ATCGAATTCACTTGCTCTGTGCAGATTCAGA...3' (SEQ. ID. No. 10)

Following amplification, the PCR products were digested with *Bam*HI and *Eco*RI and subcloned into the plasmid pUC9 which had been similarly treated. Recombinant clones were identified and sequenced. The plasmids containing recombinant α- and β-chain genes were designated p26416 and p26417 respectively. Sequence analysis of the α chain revealed a point mutation (G to T) at base 649 of the published sequence. This mutation results in a valine to leucine substitution at residue 217 of the full length mature gene product. No deviations from the published sequence were observed for the β-chain.

Full-length expression constructs. Plasmids p26416 and p26417 were treated with *Bam*HI and *Eco*RI. Fragments corresponding to the α- and β-chains were subcloned into the expression vector p27313. Recombinant clones were identified by restriction analysis and given the designations p27317 (α-chain) and p27316 (β-chain).

ΔTM expression constructs. PCR amplification of truncated α- and β-chain genes was performed using the PCR primer pairs previously described. The plasmids p26416 and p26417 were used as target DNA for the α- and β-chain, respectively. The PCR products were treated with *Bam*HI and *Eco*RI and ligated into plasmid p27316 which had been treated with the same enzymes to remove the full-length β-chain. The resulting plasmids were designated p26495 (α-ΔTM) and p26496 (β-ΔTM).

## EXAMPLE 2

### Expression of MHC Class II Molecules in *E. coli*

Construction of Host Strain W3110/DE3. *E. coli* strain W3110 was made lysogenic for the phage lambda-DE3 (which carries a copy of the T7 RNA polymerase gene) using the DE3 lysogenization kit from Novagen, following the manufacturer's instructions.

Induction of Recombinant Clones. Plasmids p27316, p27317, p26495 and p26496 were transformed into the host strain W3110/DE3.

Cultures were grown at 37°C in LB containing 0.4% glucose and 100 μg/ml ampicillin. Cells were induced in mid-log growth by addition of isopropyl-β-b-D-thio-galactopyranoside (IPTG) (0.4 mM final concentration) and allowed to grow at 37°C. Periodic samples were taken and chilled on ice prior to processing.

The α-chain clone used herein contains a nucleotide substitution at base 649, compared to the published sequence. This difference results in the substitution of a leucine residue in place of a valine residue at amino acid 217 of the full length product. This residue is within the transmembrane portion of the molecule and is therefore not present in the ΔTM construct. Because of the conservative nature of the leucine for valine substitution, and its positioning within the transmembrane region, the mutation was not considered a significant hinder to further experiments with regard to peptide binding and interaction with T-cells.

Induction of both full-length and truncated constructs in W3110/DE3 resulted in the substantial accumulation of protein at or near the expected size as evaluated by SDS-PAGE. Below is a summary of the lengths and expected molecular weights for each of the four proteins evaluated:

significant binding of MBP(83-102)$Y^{83}$ peptide. In addition, recombinant chains showed increased binding as compared to equimolar amount of DR2 native heterodimer. The results were reproducible in 4 different experiments. The specificity of the peptide binding was demonstrated by incubating the chains with an equivalent amount of another epitope from the same myelin basic protein, MBP(1-14). In all cases, the binding of MBP(1-14) was insignificant.

Association and Dissociation kinetics of rDR2 chains with radiolabeled peptides. The on rate kinetics of binding was measured similarly as described above. Chains at a concentration of 200 $\mu$g/ml were incubated at 37°C with labeled peptide. At various times, 15 $\mu$l of sample was removed, chilled to 4°C and analyzed on 13.5% SDS-PAGE. The percent peptide occupancy was calculated from the specific activity as described above. The stability of chain-peptide complexes were compared at zero and at 37°C (Figure 2). Single chain-peptide complexes appeared to be as stable as the heterodimeric native DR2-peptide complexes.

## EXAMPLE 4

### Peptide Binding Assay with Biotinylated Antigenic Peptide

Further binding studies were carried out using the four recombinant chains, rDR2$\alpha$(+TM), rDR2$\alpha$(-TM), rDR2$\beta$(+TM), rDR2$\beta$(-TM), purified by conventional preparative chromatographic procedures as described above. Biotinylated-MBP (83-102)$Y^{83}$, biotinylated-MBP (124-143) and biotinylated-MBP (1-14) peptides were used for the binding assay. Recombinant chains at a concentration of 0.2 mg/ml was incubated with 50 fold molar excess of biotinylated-MBP peptides. For the quantitation of the percentage of chain occupied with the biotinylated-peptide, resulting complexes were analyzed in a plate assay using enzyme conjugated avidin system. One mg per 50 ml affinity purified L243 monoclonal antibody, polyclonal anti-alpha and polyclonal anti-beta were coated for the DR2, $\alpha$ and $\beta$ chains (with and without transmembrane regions), respectively on a 96 well microtiter plate. The polyclonal anti-$\alpha$ and anti-$\beta$ antibodies were purified from immunized rabbit sera on a antigen-coupled sepharose-4B column. Calibration of the assay was achieved by coating know amounts of biotinylated-BSA. After capture of complexes, unbound peptide was removed by washing, followed by incubation with avidin-alkaline phosphatase. Unbound enzyme

as a substrate. The reaction was stopped by 2 N sulfuric acid at 5 min. and the absorbance was measured at 450 nm.

The increase in gamma IFN production of T cells has been shown to occur following TCR occupancy by specific ligands. The complexes of native DR2 with MBP (83-102)$Y^{83}$ peptide were used as a positive control in this assay. The specificity of the increase in gamma IFN production was demonstrated by complexes of native DR2 or chains with irrelevant high affinity MBP (124-143) peptide in all experiments. Similarly, complexes of DR3 with MBP (83-102)$Y^{83}$ peptide was used to demonstrate the restriction of SS8T cloned T cells by HLA-DR2. Results obtained with complexes of alpha chain (with or without Tm) and beta chain (with or without Tm) are presented in Figure 4 and Figure 5.

These results clearly demonstrate that MHC class II single chain-peptide complexes function like complexes of antigenic peptide and native heterodimer.

## EXAMPLE 6

Treatment of EAE using recombinantly produced I-$A^s$ $\alpha$ chain

This example demonstrates the ability of the recombinantly produced single chain complexes of the invention to induce anergy *in vivo*. These experiments demonstrate prevention of EAE in SJL/J mice. The $\alpha$ chains of $IA^s$ were recombinantly expressed using the methods of the invention. Briefly, PCR primers were prepared based on the gene sequence available in Genbank to isolate the gene from mouse spleen cells. The resultant gene was expressed using expression vector p27313, as described above.

EAE was induced by adoptive transfer of 1 x $10^7$ MBP(91-103) reactive T cells as described in Sharma *et al. Proc. Natl. Acad. Sci. USA* 88:11465-11469 (1991). The experiment was performed using $\alpha$ chain of $IA^s$ complexed with MBP 91-103 or 1-14 prepared as described above. On days 0, 2, 4, and 6, each mouse received 40$\mu$g of complexes in as described in Sharma *et al.* The results are shown in Figure 6. As can be seen there, animals receiving PBS alone or irrelevant complex (I-$A^s$ complexed with MBP(1-14)) showed paralysis, whereas animals receiving relevant complex (I-$A^s$ complexed with MBP(91-103)) did not.

Ubi "top" primer

BamHI

5'...TCAGGATCCGATCGTGGAGGATGATTAAATGCAAATTTTTGTCAAGACTTT GACTGGT ...3' (SEQ. ID. No. 16)

The primer sequence includes the phi-10 coupler region and the underlined sequence represents the actual ubiquitin 5' sequence.

Ubi "reverse" primer

EcoRI SacII

5'... TGAATTCCCGCGGAGTCTCAAGACTAAGTGCAAAGTGGA...3' (SEQ. ID. No. 17)

The unique SacII site is generated by altering the base sequence so as to create the restriction site for fusion cloning but maintaining the amino acid sequence of the molecule.

An expression vector for production of desired fusion products, p27340, was generated by digesting p27313, described above, with BamHI and EcoRI and ligating the 5500 bp fragment with the 259 bp ubi-76 PCR product digested with the same enzymes.

p27340 which can be used to fuse any gene to the ubiquitin gene so as to make a fusion product which can then be specifically cleaved to yield a protein of desired N-terminus. The vector is selectable with ampicillin.

The nucleic acid encoding the DR alpha chain lacking its transmembrane region was cloned in p27340 and was expressed as a fusion product of expected molecular weight of 30,758 daltons. SDS-PAGE gels showed the presence of a double band similiar to the expression products from plasmid p26495.

Construction of p27351 and p27373. The DR alpha chain sequences were amplified using PCR to generate the full length sequence as well as the sequence lacking the transmembrane and cytoplasmic domains. The PCR primers designed for the fusion of these sequences to the ubiquitin 76 sequence were as follows :

The plasmid was transformed into the expression host W3110/DE3. Following growth and IPTG induction, expression of the fusion protein of Ubiquitin 76 + DR alpha F/L of the expected molecular weight $M_r$ = 34,625 was observed.

## EXAMPLE 8

### Additional E. coli MHC II Expression Plasmids

Construction of p28524 for expression of DR alpha ΔTM chain that is shorter by 10 residues. The plasmid p26495 expresses DR alphaΔTM chain in the pET expression system. Upon IPTG inductions, the SDS-PAGE gel shows a doublet of bands at the expected molecular weight. These bands were sequenced for the first 5 N-terminal residues and both gave the correct sequence for the alpha chain. Plasmid p28524 was constructed to generate a even more truncated version of DR alphaΔTM chain. The sequence was PCR amplified using the following primers:

Top strand primer:

5'...CGGGATCCGATCGTGGAGGATGATTAAATGATCAAAGAAGAACATGTGATC AT...3' (SEQ. ID. No. 22)

Bottom strand primer:

5'...ATCGAATTCTTAAGCATCAAACTCCCAGTGCTT...3' (SEQ. ID. No. 23)

The PCR product was digested with BamH1 and EcoR1 and cloned into p27313 digested with the same enzymes.

Construction of E.coli expression plasmid with tetracycline resistance. The following plasmids were constructed for expression of MHC class II single chains in E.coli. Plasmids p26495, p26496, p27316 and p27317 described above express ΔTM and full-length DR alpha and beta chains in the presence of ampicillin resistance. For scale-up culturing of E.coli strains, ampicillin is not an effective antibiotic as it is degraded rapidly due to the β-lactamase secreted by the cells containing amp resistant plasmids. Therefore tetracycline resistance gene was cloned into the above plasmids to make them more stable under fermentation conditions.

Construction of p27329 and p27330. The tetracycline resistance gene was amplified by PCR using pBR322 as target DNA and the following PCR primers:

The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference.

```
Thr Thr Gly Val Ser Glu Thr Val Phe Leu Pro Arg Glu Asp His Leu
    130                 135                 140

Phe Arg Lys Phe His Tyr Leu Pro Phe Leu Pro Ser Thr Glu Asp Val
145                 150                 155                 160

Tyr Asp Cys Arg Val Glu His Trp Gly Leu Asp Glu Pro Leu Leu Lys
                165                 170                 175

His Trp Glu Phe Asp Ala Pro Ser Pro Leu Pro Glu Thr Thr Glu Asn
            180                 185                 190

Val Val Cys Ala Leu Gly Leu Thr Val Gly Leu Val Gly Ile Ile Ile
        195                 200                 205

Gly Thr Ile Phe Ile Ile Lys Gly Val Arg Lys Ser Asn Ala Ala Glu
    210                 215                 220

Arg Arg Gly Pro Leu
225
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 714 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..714

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 595..714
(D) OTHER INFORMATION: /note= "Encodes the trans-membrane region within HLA DR2-Dw2 Beta Chain."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
GGG GAC ACC CGA CCA CGT TTC TTG CAG CAG GAT AAG TAT GAG TGT CAT        48
 ly Asp Thr Arg Pro Arg Phe Leu Gln Gln Asp Lys Tyr Glu Cys His
  1               5                  10                  15

TTC TTC AAC GGG ACG GAG CGG GTG CGG TTC CTG CAC AGA GAC ATC TAT        96
Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu His Arg Asp Ile Tyr
            20                  25                  30

AAC CAA GAG GAG GAC TTG CGC TTC GAC AGC GAC GTG GGG GAG TAC CGG       144
Asn Gln Glu Glu Asp Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg
        35                  40                  45

GCG GTG ACG GAG CTG GGG CGG CCT GAC GCT GAG TAC TGG AAC AGC CAG       192
Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln
    50                  55                  60

AAG GAC TTC CTG GAA GAC AGG CGC GCC GCG GTG GAC ACC TAC TGC AGA       240
Lys Asp Phe Leu Glu Asp Arg Arg Ala Ala Val Asp Thr Tyr Cys Arg
65                  70                  75                  80
```

```
                    85                  90                  95

Pro Lys Val Thr Val Tyr Pro Ala Arg Thr Gln Thr Leu Gln His His
            100                 105                 110

Asn Leu Leu Val Cys Ser Val Ser Gly Phe Tyr Pro Ala Ser Ile Glu
        115                 120                 125

Val Arg Trp Phe Arg Asn Ser Gln Glu Glu Lys Ala Gly Val Val Ser
    130                 135                 140

Thr Gly Leu Ile Gln Asn Gly Asp Trp Thr Phe Gln Thr Leu Val Met
145                 150                 155                 160

Leu Glu Thr Val Pro Arg Ser Gly Glu Val Tyr Thr Cys Gln Val Glu
                165                 170                 175

His Pro Ser Val Thr Ser Pro Leu Thr Val Glu Trp Arg Ala Gln Ser
            180                 185                 190

Glu Ser Ala Gln Ser Lys Met Leu Ser Gly Val Gly Gly Phe Val Leu
        195                 200                 205

Gly Leu Leu Phe Leu Gly Ala Gly Leu Phe Ile Tyr Phe Lys Asn Gln
    210                 215                 220

Lys Gly His Ser Gly Leu His Pro Thr Gly Leu Val Ser
225                 230                 235
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CGGAATTCCG 10

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 58 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TCAGGATCCG ATCGTGGAGG ATGATTAAAT GCAAATTTTT GTCAAGACTT TGACTGGT 58

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATCGAATTCT TAAGCATCAA ACTCCCAGTG CTT 33

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGGGATCCGA TCGTGGAGGA TGATTAAATG ATCAAAGAAG AACATGTGAT CAT 53

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ATCGAATTCT TAAGCATCAA ACTCCCAGTG CTT 33

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATCTCGAGTT TGACAGCTTA TCATCG 26

WHAT IS CLAIMED IS:

1. A composition comprising a recombinant MHC polypeptide which binds an antigenic peptide and which has altered glycosylation.

2. A composition of claim 1, wherein the MHC polypeptide lacks a transmembrane domain.

3. A composition of claim 1, wherein the MHC polypeptide is expressed by a prokaryotic host cell comprising an expression vector containing a nucleotide sequence encoding the MHC polypeptide.

4. A composition of claim 3, wherein the prokaryotic cell is *E. coli*.

5. A composition of claim 1, further comprising a second MHC polypeptide associated with the MHC polypeptide, thereby forming a heterodimeric MHC molecule.

6. A composition of claim 1, wherein the polypeptide is encoded by a sequence from an MHC class II gene.

7. A composition of claim 1, wherein the polypeptide is a $\beta$ subunit of an MHC class II molecule.

8. A method of producing an MHC polypeptide, the method comprising:

a) growing in culture a prokaryotic cell containing an expression vector comprising a nucleotide sequence encoding the MHC polypeptide under conditions such that the polypeptide is expressed; and

b) isolating the MHC polypeptide.

9. A method of claim 7, wherein the cell comprises nucleotide sequences encoding two MHC polypeptides.

22. A complex of claim 17, wherein an epitope on the peptide is recognized by an autoreactive T cell associated with multiple sclerosis, rheumatoid arthritis, or myasthenia gravis.

23. A complex of claim 17, wherein the peptide comprises residues 138-167 of human AChR $\alpha$ subunit, residues 84-102 of human MBP, or residues 148-162 of human MBP.

24. A complex of claim 17 wherein, the MHC polypeptide is Class II MHC.

25. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the MHC-peptide complex of claim 17.

26. A pharmaceutical composition of claim 25, wherein the complex is embedded in a liposome.

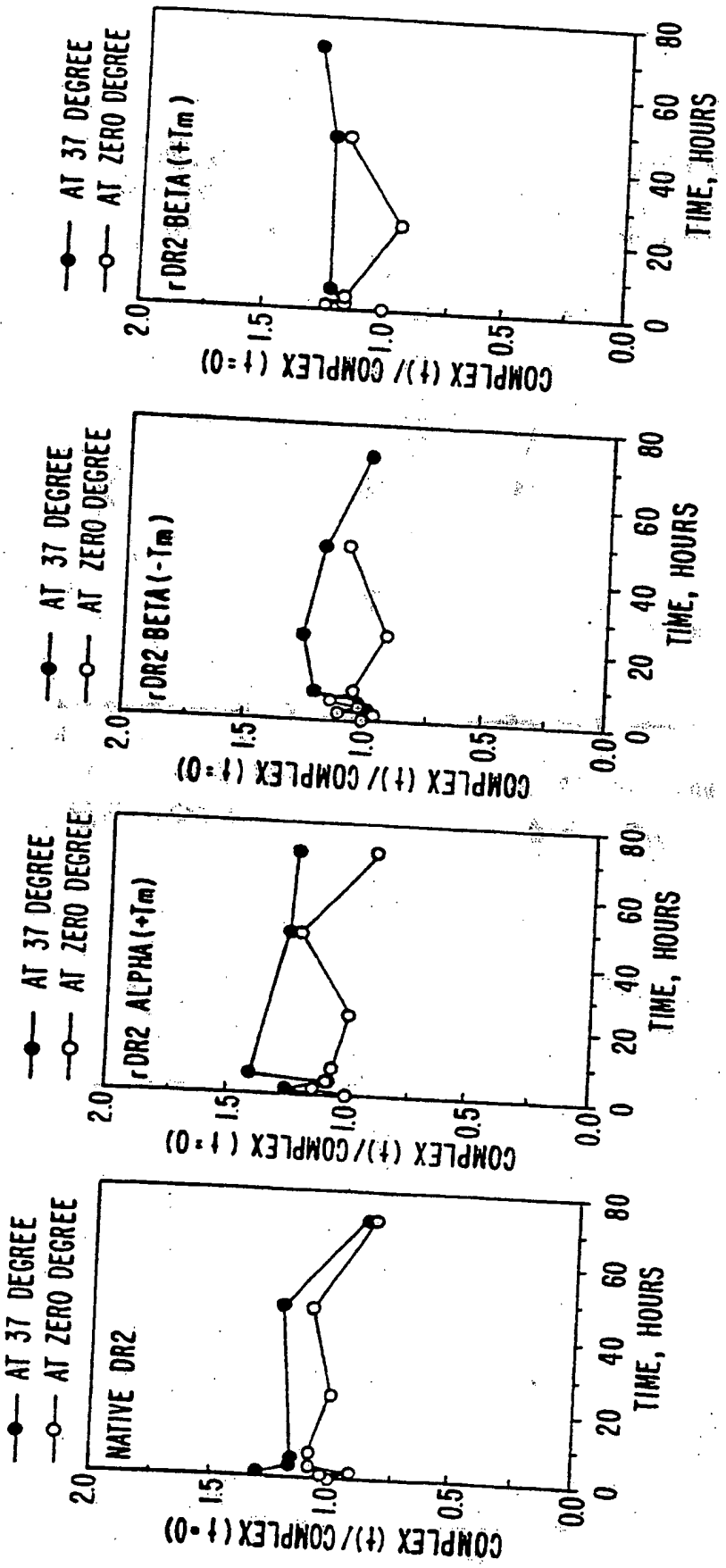
AT 37 DEGREE
AT ZERO DEGREE
rDR2 BETA (+Tm)
rDR2 BETA (-Tm)
rDR2 ALPHA (+Tm)
NATIVE DR2
COMPLEX (t)/ COMPLEX (t=0)
TIME, HOURS
0.0
0.5
1.0
1.5
2.0
0
20
40
60
80

FIG. 2.

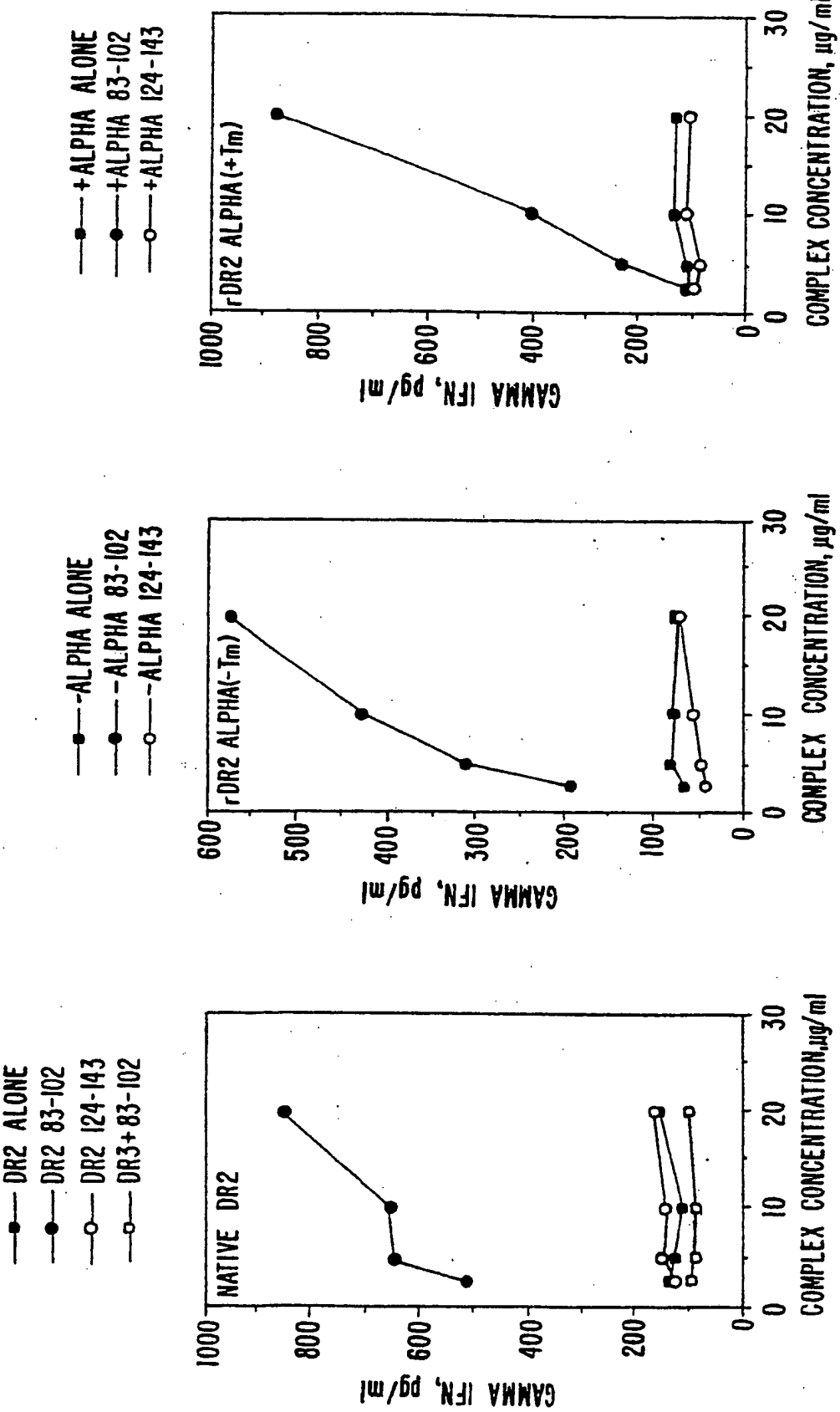
DR2 ALONE
DR2 83-102
DR2 124-143
DR3+ 83-102
NATIVE DR2
GAMMA IFN, pg/ml
COMPLEX CONCENTRATION, µg/ml
-ALPHA ALONE
-ALPHA 83-102
-ALPHA 124-143
rDR2 ALPHA(-Tm)
+ALPHA ALONE
+ALPHA 83-102
+ALPHA 124-143
rDR2 ALPHA(+Tm)

FIG. 4.

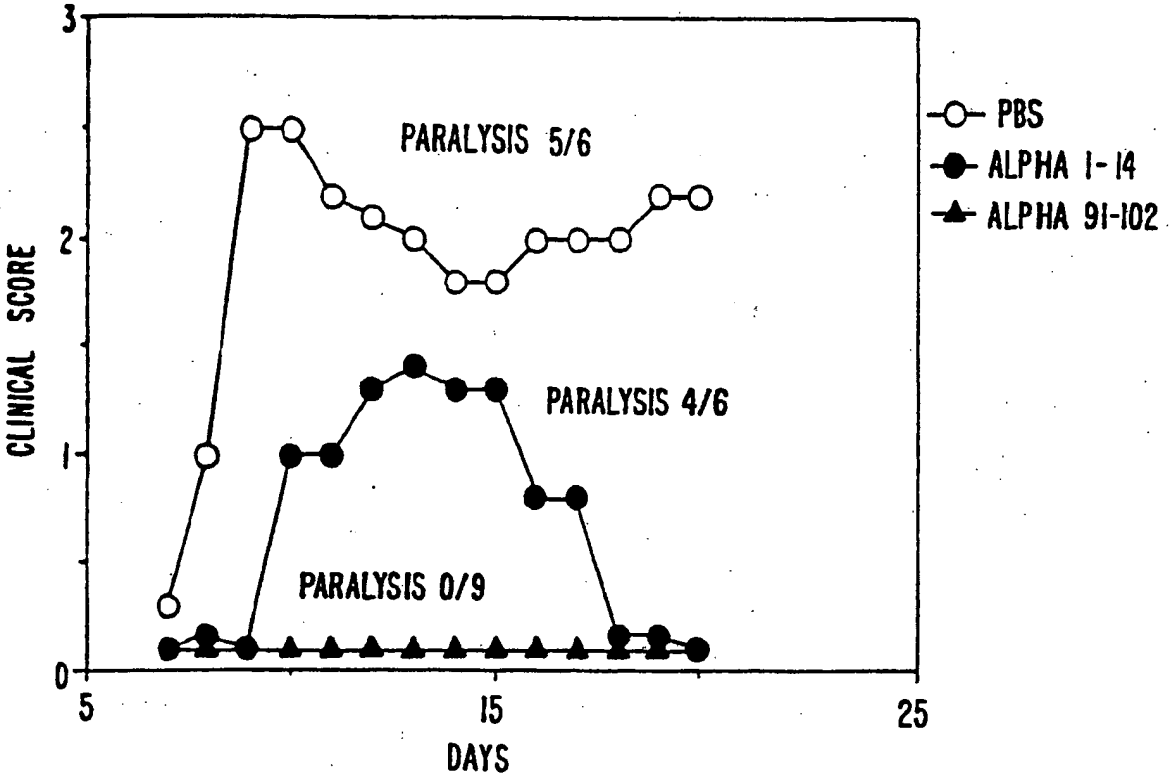
3
2
1
0
CLINICAL SCORE
PARALYSIS 5/6
PARALYSIS 4/6
PARALYSIS 0/9
5
15
25
DAYS
PBS
ALPHA 1-14
ALPHA 91-102

FIG. 6.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | Nature, volume 353, issued 12 September 1991, R. N. Germain et al., "MHC class II structure, occupancy and surface expression determined by post-endoplasmic reticulum antigen binding", pages 134-139, see abstract. | 1-26 |
| Y | Proceedings of the National Academy of Sciences USA, volume 89, issued December 1992, M. R. Jackson et al., "Empty and peptide-containing conformers of class I major histocompatibility complex molecules expressed in Drosophila melanogaster cells", pages 12117-12121, see abstract. | 1-26 |